REMARKS

The Examiner has indicated that the amendment mailed September 17, 2001 has been entered and Claims 14 and 17-28 are pending and under examination. Applicants note that the Examiner has indicated Claims 20-22 are allowed. Applicants also appreciatively note that the Examiner has removed the previously made prior art rejections. However, Claims 14, 17-19, and 23-28 stand rejected. Applicants have cancelled Claims 14 and 26-28 without prejudice and added new Claims 29-32, which correspond to the cancelled Claims. As the newly added Claims correspond to the cancelled Claims, the rejections of Claims 14 and 26-28 are addressed as they may pertain to the newly added Claims. The Examiner's rejections are addressed below in the following order:

1) Claim 23 stands rejected under 35 U.S.C. §112, second paragraph as allegedly being indefinite;

2) Claims 14, 18-19, and 24-28 stand rejected under 35 U.S.C. §112, first paragraph as allegedly not meeting the written description requirement; Claim 18 is alleged to contain new matter;

3) Claims 14 and 23-27 stand rejected under 35 U.S.C. §112, first paragraph, as allegedly not meeting the written description requirement; and

4) Claims 14, 17-18 and 28 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Garman *et al.* in view of Macatonia et al., Mehta-Damani *et al.* or Takamizawa *et al.*

1) Claim 23 is Definite

The Examiner has rejected Claim 23 under 35 U.S.C. §112, second paragraph as allegedly being indefinite. In particular, the Examiner indicates that there is no antecedent basis for "the protein" in lines 1 and 3 of the Claim. Applicants have amended Claim 23 recite "microbial subtilisin" in place of "the protein" in this Claim. Thus, Applicants submit that this Claim is in proper form and respectfully request that this rejection be withdrawn.

2-3) The Written Description Requirement is Met

The Examiner has rejected Claims 14, 18-19 and 24-28 under 35 U.S.C. §112, first paragraph as allegedly not meeting the written description requirement; Claim 18 is alleged to contain new matter. Claims 14 and 23-27 also stand rejected under 35 U.S.C. §112, first paragraph, as allegedly not meeting the written description requirement.

In particular, the Examiner argues that "Specifically, the only disclosure of adherent monocyte-derived dendritic cells was provided in Example 1 (page 26, lines 1-8). . . . Claim 18 does not require that the adherent monocyte derive [sic] dendritic cell of part (a)(I) and the naïve T-cell part of (a)(ii) be from the same naïve individual (i.e. a single blood source), applicant's claimed method encompasses embodiments, with respect to the source of the dendritic cell, which were not originally contemplated by applicant." (Office Action, page 2, emphasis original). In order to further the prosecution of the present application and their business interests, yet without acquiescing to the Examiner's arguments, Applicants have amended Claim 18 to recite that the dendritic cell and naïve T-cell are from the same source. Thus, Applicants respectfully request that this rejection be withdrawn. Applicants also submit that dependent Claim 19 is likewise allowable.

In regard to Claims 14 and 23-27, the Examiner argues that Applicants have not adequately described the sequences from the protein/protease of interest that are sequences which produce an altered or lesser allergenic response. In particular, the Examiner argues that "Applicant has not identified what are such homologs in terms of structure which would differentiate them from homologous [sic] which produce an allergenic response . . . There is no disclosure of the distinguishing attributes (even highly homologous sequences could still induce T-cell responses if they retain a motif which is recognized by MHC and T-cell receptors) of the genus. Structural features that would distinguish the sequences of the genus from other homologous sequences have not been dislosed." (Office Action, page 3). Applicants must respectfully traverse the Examiner's rejections.

Applicants respectfully submit that the presently claimed *methods* are more than sufficiently supported by the Specification as filed. Applicants note that in order to correct typographical inconsistencies and provide proper Claim order, Applicants have cancelled Claims 14, and 26-28 without prejudice. Applicants have added new Claims 29-32, which correspond to the cancelled Claims. Support for these Claims is found in the Claims as filed, as well as within the Specification as filed (*See e.g.,* Examples 1-2).

The law indicates that "the 'essential goal' of the description of the invention is to clearly convey the information that an applicant has invented the subject matter which is claimed" (*In re Barker*, 559 F.2d 588, 592 n.4, 194 USPQ 470,473 n.4 (CCPA 1977)), as well as to put the public in possession of what the applicant claims as the invention (*See, Regents of the University of California v. Eli Lilly*, 119 F.3d 1559, 1566, 43 USPQ2d 1398, 1404 (Fed. Cir. 1997), *cert. denied*, 523 U.S. 1089 (1998). However, "[i]f a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate description requirement is met." (MPEP 2163; *See also, Vas-Cath Inc., v. Makurhar*, 935 F.2d at 1563, 19 USPQ2d at 1116 (Fed. Cir. 1991); and *Martin v. Johnson*, 454 F.2d 746, 751, 172 USPQ 391, 395 (CCPA 1972)).

Applicants submit that the Examiner has apparently misapprehended the presently claimed invention. Indeed, it is through the use of the methods that the user of the presently claimed invention determines the sequences that can be modified in order to reduce the allergenic response to particular proteins. The claimed steps result in the production of modified proteins (*i.e.,* via identification of epitopes) that only induce T-cell responses that are either less than or substantially equal to baseline values. The present invention provides means to identify T-cell epitopes that may be modified so to provide proteins with reduced allergenicity. Thus, by performing the steps of the claimed methods, the Examiner's questions are answered with regard to the epitopes that are suitable for modification.

Applicants provide working Examples showing the use of the method of the presently claimed invention. In these Examples, descriptions of the preparation of the cells and peptide antigens are provided, as well as the immune response observed following the exposure of the T-cells to the prepared epitopes (in the presence of DCs).

As described in the Examples, the peptides to be tested are based on the sequence to be analyzed (*See*, Figures 6-8, for the specific embodiment described). The immunogenic response (*i.e.*, T-cell proliferation) to the prepared peptides is observed and assessed (See, Figures 4-5, and 9-10, for the specific embodiment described). As indicated in Example 2, the results obtained upon performing the steps of the Examples confirm that, by practicing the presently claimed methods, it is possible to determine which epitopes are capable of inducing a T-cell response that is less than or substantially equal to the baseline response level. Furthermore, the Example describes an alanine substitution assay to determine the role of any specific residue within the epitope. Thus, Applicants provide sufficient teaching that one of ordinary skill in the art could take and use the presently claimed invention for their intended purpose (*i.e.*, the presently claimed methods are suitable for the determination of any T-cell epitopes that are suitable for modification in order to reduce allergenicity). Of course, Applicants are not limited by the specific embodiment used to demonstrate their invention, as set forth in the Examples.

In addition, the Specification as filed, contains descriptions and definitions as to variants, equivalent residues, homology, etc. (*See*, pages 14-17). Thus, Applicants respectfully submit that the written description of the Specification as filed, is more than sufficient to support the presently claimed invention and request that this rejection be withdrawn.

4) The Claims are Unobvious

The Examiner has rejected Claims 14, 17-18 and 28 under 35 U.S.C. §103(a), as allegedly being unpatentable over Garman *et al.*, in view of Macatonia *et al.*, Mehta-Damani *et al.* or Takamizawa *et al.*

The Examiner argues that Garman *et al.* teach the "identification of T-cell epitopes within a protein allergen and modification thereof (via substitution of amino acid residues) to provide peptides which induce a lowered or not any proliferative response of T-cells . . ." (Office Action, page 4). Applicants note that the Examiner admits that Garman *et al.* fail to teach the use of naïve T-cells. Rather, as indicated by the

Examiner, Garman *et al.* teach epitope screening with T-cells from sensitized individuals. (Office Action, page 4).

The Examiner further argues that "[e]ach of the secondary references (all cited on From [sic] 1449) teaches that one can obtain human blood samples and derive dendritic cells (DCs) and naïve T-cells therefrom such that the DCs can present antigen to the naïve T-cells to induce a proliferative response." (Office Action, page 5). The Examiner argues that "[I]t would have been obvious to identify epitopes within the allergen of Garman et al. by using DCs and naïve T-cells from a blood sample, as taught by the secondary references. Motivation to do so would have been to conduct tests using blood cells from non-sensitized individual [sic] so that one would not need to find patients with the allergic disorder." (Office Action, page 5).

The Examiner indicates that Applicants' comments in the previous Office Action Response mailed April 30, 2001, are directed to how the authors of the three cited references prepared their DCs and the maturity of the DCs obtained, yet the Claims do not indicate anything about the maturity of the DCs. However, Applicants point out that the present Claims provide an indication of the status of the DCs used in the methods, as the DCs are combined with cytokines that result in the differentiation of the DCs prior to their combination with the T-cells.

A. There is No *Prima Facie* Case Of Obviousness

A *prima facie* case of obviousness requires the Examiner to cite to a combination of references which (a) suggests or motivates one of skill in the art to modify their teachings to yield the claimed invention, (b) discloses the elements of the claimed invention, **and** (c) provides a reasonable expectation of success should the claimed invention be carried out. Failure to establish **any** one of these requirements precludes a finding of a *prima facie* case of obviousness and, without more, entitles Applicants to withdrawal of the rejection of the claims in issue.[2] Applicants urge that the Examiner has failed to establish not one, but **all three** requirements as discussed below.

1. The Combined References Fail To Disclose All The Limitations Of The Claims

It is axiomatic for establishing a *prima facie* case of obviousness that "all the claim limitations must be taught or suggested by the prior art."[3] However, the Examiner has not established that the combined references disclose the all of the limitations set forth in the pending Claims, including obtaining human dendritic and naïve CD4+ and/or CD8+ cells from a single source, differentiating the dendritic cells, combining the differentiated dendritic cells with the CD4+ and/or CD8+ cells, and a peptide of interest. Indeed, Applicants respectfully submit that none of the requirements for a showing of obviousness has been made. To the contrary, the references cited by the Examiner highlight the unobvious nature of the presently claimed invention.

a. The Garman *et al.* Reference Fails To Disclose All The Claims' Limitations

With respect to each of the rejected Claims 14, 17-18 and 28, Applicants previously argued that this reference fails to teach the use of naïve T-cells, a fact with which the Examiner agrees (Office Action, page 4). Thus, the primary reference does not teach each limitation of the pending Claims.

b. The Macatonia *et al.* Reference Does Not Provide The Elements Which Are Absent From Garman *et al.*

Applicants respectfully submit that the Macatonia *et al.* reference does not bridge the gaps of Garman *et al.* For example, neither this reference, nor Garman *et al.* teach the limitation of differentiating the DCs prior to their combination with T-cells. In contrast to the presently claimed invention, the Macatonia *et al.* reference indicates that the DCs

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2 *See e.g., Northern Telecom Inc. v. Datapoint Corp.*, 15 USPQ2d 1321, 1323 (Fed. Cir. 1990); and *In re Dow Chemical Co.*, 837 F.2d 469, 5 USPQ2d 1529 (Fed. Cir. 1988).

3 MPEP § 2143.03, citing *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974).

are pulsed with HIV, HIV peptides or recombinant gp120 (*See*, page 401) and are then combined with T-cells. The presently claimed method involves differentiating the DCs by exposing them to cytokines (*See e.g.*, page 26, lines 1-11), prior to combining them with T-cells. There is no suggestion in either the Macatonia *et al.*, reference, or the Garman *et al.* reference that such a differentiation step be conducted. Thus, these references, taken alone or in combination do NOT teach nor suggest the presently claimed invention. Nonetheless, in order to further their business interests and the prosecution of the present application, yet without acquiescing to the Examiner's arguments, Applicants have amended independent Claims 17 and 18 to more clearly describe the differentiation step of the present invention. Applicants reserve the right to prosecute the originally filed and/or similar Claims in the future.

c. The Mehta-Damani *et al.* Reference Fails To Supplement The Missing Limitations

Applicants also respectfully submit that the Mehta-Damani *et al.* reference does not teach all of the limitations of the pending Claims. Mehta-Damani *et al.* teach an *in vitro* system for generating antigen-specific CD4+ T-cells from previously umprimed individuals. As described in this reference, the method utilizes DCs (and macrophages, in some embodiments) and T-cells isolated from human peripheral blood (*See*, page 1207). As with the Garman *et al.* and Macatonia *et al.* references, but unlike the presently claimed invention, the method of Mehta-Damani does not involve a step in which the DCs are differentiated. Rather, the purified DCs obtained from human peripheral blood are irradiated and then added to CD4+ T-cells in culture. Thus, there is no teaching in Mehta-Damani that would motivate one of skill in the art to treat the DCs so as to promote their differentiation, prior to their use as antigen presenting cells for T-cells. Therefore, Applicants respectfully submit that this reference, taken alone, in combination with Garman, or in combination with any of the other cited references, does not teach nor motivate one to produce the presently claimed invention.

d. The Takamizawa *et al.* Reference Fails to Provide the Missing Elements of the Pending Claims

As with the Garman *et al.*, Macatonia *et al.*, and Mehta-Damani *et al.* references, Applicants respectfully submit that the Takamizawa *et al.* reference does not teach the presently claimed invention, either alone or in combination with any of the other references. Takamizawa *et al.* teach the use of two populations of DCs to present antigens to T-cells. Their data indicate that "differentiated DC can be obtained from a population of HLA-DR^{bright}, lineage-negative, CD2^{+} cells present in peripheral blood, and that the DC derived from these DCp (DC precursors), but not DC derived from precursors that lack CD2 expression (CD2^{-} DCp), present nominal antigens to naïve T cells." (Takamizawa *et al.*, at page 2134). In this reference, the DC precursors were differentiated by incubating the precursor cells (depleted of monocytes) in conditioned medium from PHA-activated peripheral blood mononuclear cells (PBMC). There is no teaching of differentiating DCs through the use of such compounds as GM-CSF, IL-4, TNFα, nor IL-1α (*i.e.*, the only differentiation step described in Takamizawa *et al.* requires the use of medium conditioned by incubation of PBMC stimulated by PHA.

In addition, Takamizawa *et al.* do NOT teach a method that involves the use of peptides to stimulate T-cells. Rather, Takamizawa *et al.* teach the use of KLH or HIV gp160 antigens, which are clearly not "peptides." There is no indication that the use of peptides, rather than these large molecules, would successfully result in a T-cell response in the presence of DCs. Thus, Takamizawa *et al.* do not teach the presently claimed invention, in which peptides are used in conjunction with differentiated DCs to induce a T-cell response. Thus, Takamizawa *et al.*, taken alone or in combination with any of the above references does not teach the presently claimed invention. Indeed, Applicants submit that particularly when viewed as a whole, the presently claimed invention is unobvious over the prior art[4]. In addition, Applicants respectfully submit that

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[4] "[T]he question under 35 U.S.C. § 103 is not whether the differences *themselves* would have been obvious. Consideration of differences, like each of the findings set forth in *Graham*, is but an aid in reaching the ultimate determination of whether the claimed invention *as a whole* would have been obvious." *Stratoflex Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 1537, 218 USPQ 871 (Fed. Cir. 1983) (*emphasis original*).

as indicated in *Intel Corp. v. United States Int'l Trade Comm'n*, 946 F.2d 821, 842, 20 USPQ2d 1161, 1179 (Fed. Cir. 1991):

> Claim limitations may, and often do, read on the prior art, particularly in combination patents. That all elements of an invention may have been old (the normal situation), or some old and some new, or all new, is however, simply irrelevant. Virtually all inventions are combinations and virtually all are combinations of old elements. [Quoting from *Environmental Designs, Ltd. v. Union Oil Co.*, 713 F.2d 693, 698, 218 USPQ 865, 870 (Fed. Cir. 1983)].

Thus, *even if* (which Applicants submit they are not) the elements of the presently claimed invention are described in the prior art, Applicants respectfully submit that the invention itself is not described, taught, nor suggested in any of the cited references, taken alone or in combination. Thus, Applicants respectfully submit that this rejection should be withdrawn.

2. Motivation To Practice The Recited Combination Of Steps

An essential requirement for a *prima facie* case of obviousness is whether a person skilled in the art would be **motivated** to modify the reference to arrive at the **claimed invention**.[5] In particular,

> "the examiner must show *reasons* that the skilled artisan, confronted with the same problems as the inventor and with no knowledge of the *claimed invention*, would select the elements from the cited prior art references for combination in the manner claimed."[6]

Such evidence is lacking in the present Office Action, as none of the references, taken alone or in combination suggest using cytokines to differentiate the DCs, prior to the combination of the DCs with the T-cells, as presently claimed. The Examiner has provided no references nor reasons why one of skill in the art would take the cited combination of references, and then modify the methods so as to differentiate the DCs

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5 *In re Fine*, 837 F.2d 1071, 1074, 5 USPQ2d 1596, 1598-99 (Fed. Cir. 1988); and *In re Jones*, 21 USPQ2d 1941, 1943 (Fed. Cir. 1992).

6 *In re Rouffet*, 47 USPQ2d 1453 (Fed. Cir. 1998); *Robotic Vision Systems Inc. v. View Engineering Inc.*, 51 USPQ2d 1948 (Fed. Cir. 1999), (*emphasis added*).

using cytokines, prior to their use in an assay system for methods for determining T-cell epitopes or reducing the allergenicity of proteins, as presently claimed. Nor does the Examiner point to any reference that teaches the combination of differentiated DCs and exposure of CD4+ and/or CD8+ T-cells to peptides in the presence of differentiated DCs, such that the claimed methods for determining a T-cell epitope of a peptide, nor methods for reducing the allergenicity of proteins are produced. Indeed, Applicants submit that there is nothing in the prior art to lead a person of ordinary skill to the combination of the teachings of the these references to design the claimed methods of the present iinvention other than the hindsight knowledge of Applicants' methods. Furthermore, "[T]he motivation to combine references cannot come from the invention itself." (*Heidelberger Druckmaschinen v. Hantscho Commercial Products*, 30 USPQ2d 1377, 1380, 21 F.3d 1068 (Fed. Cir. 1994)). Thus, Applicants respectfully submit that this prong of the obviousness analysis is not met and request that this rejection be withdrawn.

3. A Reasonable Expectation Of Success Is Not Established

A further fundamental requisite of establishing a *prima facie* case of obviousness is that there is a reasonable expectation of success in practicing the recited method steps or producing the recited compositions.

> "[T]he reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure."[7]

There is no indication in the references cited nor the Examiner's arguments, that the cited references, taken alone or in combination, would successfully produce the presently claimed invention. As indicated above, Applicants believe that the only way to obtain the presently claimed invention would be to use the hindsight provided by the present Specification itself. This is not permissible[8].

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7 *In re Dow Chemical Co.*, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988), as cited in *In re Vaeck*, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991).

Furthermore, "[o]ne cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention." (See *In re Fine*, 837 F.2d 1071, 1075, 5 USPQ2d 1596 (Fed. Cir. 1988)). As there is no expectation of success that the combination of the teachings of the cited references would result in the presently claimed invention, this prong of the obviousness analysis is not met. Thus, Applicants respectfully request that this rejection be withdrawn.

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[8] *In re Fritch*, 972 F.2d 1260, 1266 (Fed. Cir. 1992); *See also, W. L. Gore & Assoc. v. Garlock, Inc.*, 721 F.2d 1540, 1550, 220 USPQ 303, 311 (Fed. Cir. 1983) ("To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher.").

CONCLUSION

In light of the above remarks, the Applicants believe the pending claims are in condition for allowance and issuance of a formal Notice of Allowance at an early date is respectfully requested. If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (650) 846-5838.

Respectfully submitted,

Date: 20 May 2002

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APPENDIX I

MARKED-UP VERSION OF REWRITTEN, ADDED, AND/OR CANCELLED CLAIMS

The following is a marked-up version of the Claims, pursuant to 37 C.F.R. §1.121 (c)(1)(ii), with instructions and markings showing changes made herein to the previous version of record of the Specification and Claims. Underlining denotes added text while bracketing denotes deleted text.

Please cancel Claims 14 and 26-28.

Please amend the Claims as follows:

17. (Amended) A method for determining a T-cell epitope of a peptide, comprising the steps of:

(a) obtaining from a single human blood source a solution of dendritic cells and a solution of naïve CD4+ and/or CD8+ T-cells;

(b) [promoting differentiation] <u>differentiating said dendritic cells,</u> in said solution of dendritic cells, <u>to produce a solution of differentiated dendritic cells, wherein said differentiating comprises combining said dendritic cells with at least one cytokine</u> ;

(c) combining said solution of differentiated dendritic cells and said naïve CD4+ and/or CD8+ T-cells with the peptide, said peptide comprising said T-cell epitope; and

(d) measuring proliferation of said T-cells in said step (c).

18. (Amended) A method of reducing the allergenicity of a protein comprising the steps of:

(a) identifying a T-cell epitope in said protein by

(i) contacting an adherent monocyte-derived dendritic cell <u>that has been differentiated by exposure to at least one cytokine *in vitro*</u>, with a peptide comprising said T-cell epitope; and

(ii) contacting said dendritic cell and peptide [to] <u>with</u> a naïve T-cell, <u>wherein said naïve T-cell has been obtained from the same source as said adherent</u>

monocyte-derived dendritic cell, and whereby said T-cell proliferates in response to said peptide; and

(b) modifying said protein to neutralize said T-cell epitope such that the modified protein induces less than or substantially equal the baseline proliferation of said naïve T-cells.

23. (Amended) The method according to [claim] claim 20, wherein said epitope of [the protein] said microbial subtilisin is modified by: (a) substituting the amino acid sequence of the epitope with an analogous sequence from a human homolog [to the protein of interest] of said microbial subtilisin; (b) substituting the amino acid sequence of the epitope with an analogous sequence from a non-human homolog [to the protein of interest] of said microbial subtilisin; or (c) substituting the amino acid sequence of the epitope with a sequence which substantially mimics the major tertiary structure attributes of the epitope.

24. (Amended) The method according to [claim] 23 [14], wherein the protein is a protease.

Please add the following new Claims:

29. (New) The method according to claim 18, wherein said T-cell epitope is modified by a substitution selected from the group consisting of:

(a) substituting the amino acid sequence of said T-cell epitope with an analogous sequence from a human homolog to the protein of interest;

(b) substituting the amino acid sequence of said T-cell epitope with an analogous sequence from a non-human homolog to the protein of interest; or

(c) substituting the amino acid sequence of said T-cell epitope with a sequence which substantially mimics the major tertiary structure attributes of the epitope.

30. (New) The method according to claim 29, wherein said T-cell epitope is modified by substituting the amino acid sequence of the T-cell epitope with an analogous sequence from a human homolog to the protein of interest.

31. (New) The method according to claim 29, wherein said T-cell epitope is modified by substituting the amino acid sequence of said T-cell epitope with an analogous sequence from a non-human homolog to the protein of interest.

32. (New) The method according to claim 29, wherein said T-cell epitope is modified by substituting the amino acid sequence of the epitope with a sequence which substantially mimics the major tertiary structure attributes of said T-cell epitope.

APPENDIX II
CLEAN VERSION OF THE ENTIRE SET OF PENDING CLAIMS AS AMENDED IN THIS COMMUNICATION

The following is a list of the Claims as they would appear following entry of this amendment.

17. (Amended) A method for determining a T-cell epitope of a peptide, comprising the steps of:

(a) obtaining from a single human blood source a solution of dendritic cells and a solution of naïve CD4+ and/or CD8+ T-cells;

(b) differentiating said dendritic cells, in said solution of dendritic cells, to produce a solution of differentiated dendritic cells, wherein said differentiating comprises combining said dendritic cells with at least one cytokine;

(c) combining said solution of differentiated dendritic cells and said naïve CD4+ and/or CD8+ T-cells with the peptide, said peptide comprising said T-cell epitope; and

(d) measuring proliferation of said T-cells in said step (c).

18. (Amended) A method of reducing the allergenicity of a protein comprising the steps of:

(a) identifying a T-cell epitope in said protein by

(i) contacting an adherent monocyte-derived dendritic cell that has been differentiated by exposure to at least one cytokine *in vitro*, with a peptide comprising said T-cell epitope; and

(ii) contacting said dendritic cell and peptide with a naïve T-cell, wherein said naïve T-cell has been obtained from the same source as said adherent monocyte-derived dendritic cell, and whereby said T-cell proliferates in response to said peptide; and

(b) modifying said protein to neutralize said T-cell epitope such that the modified protein induces less than or substantially equal the baseline proliferation of said naïve T-cells.

19. The method according to claim 18, wherein the protein is a protease.

20. (Amended) A method for reducing the allergenicity of a microbial subtilisin comprising the steps of:

(a) determining a T-cell epitope of said subtilisin comprising (i) obtaining from a from a single human blood source a solution of dendritic cells and a solution of naïve CD4+ and/or CD8+ T-cells; (ii) promoting differentiation in said solution of dendritic cells; combining said solution of differentiated dendritic cells and said naïve CD4+ and/or CD8+ T-cells with peptide fragments of said subtilisin; and (iv) measuring proliferation of said T-cells in said step (iii); and

(b) modifying the peptide which includes the T-cell epitope to neutralize said epitope.

21. The method according to claim 20, wherein the microbial subtilisin is derived from a *Bacillus*.

22. The method according to claim 21, wherein the *Bacillus* is selected from the group consisting of *B. lentus, B. subtilisin, B. amyloliquefaciens* and *B. licheniformis*.

23. (Amended) The method according to claim 20, wherein said epitope of said microbial subtilisin is modified by: (a) substituting the amino acid sequence of the epitope with an analogous sequence from a human homolog of said microbial subtilisin; (b) substituting the amino acid sequence of the epitope with an analogous sequence from a non-human homolog of said microbial subtilisin; or (c) substituting the amino acid sequence of the epitope with a sequence which substantially mimics the major tertiary structure attributes of the epitope.

24. (Amended) The method according to claim 23, wherein the protein is a protease.

25. The method according to claim 24, wherein the protease is a subtilisin.

29. The method according to claim 18, wherein said T-cell epitope is modified by a substitution selected from the group consisting of:

(a) substituting the amino acid sequence of said T-cell epitope with an analogous sequence from a human homolog to the protein of interest;

(b) substituting the amino acid sequence of said T-cell epitope with an analogous sequence from a non-human homolog to the protein of interest; or

(c) substituting the amino acid sequence of said T-cell epitope with a sequence which substantially mimics the major tertiary structure attributes of the epitope.

30. The method according to claim 29, wherein said T-cell epitope is modified by substituting the amino acid sequence of the T-cell epitope with an analogous sequence from a human homolog to the protein of interest.

31. The method according to claim 29, wherein said T-cell epitope is modified by substituting the amino acid sequence of said T-cell epitope with an analogous sequence from a non-human homolog to the protein of interest.

32. The method according to claim 29, wherein said T-cell epitope is modified by substituting the amino acid sequence of the epitope with a sequence which substantially mimics the major tertiary structure attributes of said T-cell epitope.